# Microbial source markers assessment in the Bogotá River basin (Colombia)

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## ABSTRACT

The microbiological indicators traditionally used to assess fecal contamination are insufficient to identify the source. The aim of this study was to detect microbial markers to identify the source of fecal pollution in the Bogotá River (Colombia). For this, we determined non-discriminating indicators such as *Escherichia coli*, somatic coliphages and phages infecting strain RYC2056 of *Bacteroides*, and potential source tracking markers as phages infecting strains GA17, HB13, and CA8 of *Bacteroides*, sorbitol-fermenting bifidobacteria, and molecular markers of *Bifidobacterium adolescentis*, *Bifiodobacterium dentium*, and *Bacteroidetes* in raw municipal wastewaters, slaughterhouse wastewaters, and the Bogotá River. Bacteriophages infecting *Bacteroides* strain GA17 and the molecular markers identified the wastewater sources. In contrast, sorbitol-fermenting bifidobacteria failed regarding specificity. In the Bogotá River, phages infecting strain GA17 were detected in all samples downstream of Bogotá, whereas they should be concentrated from 1 l samples in upstream samples containing less than $10^3$ *E. coli*/100 ml to be detected. In the river water, the fraction of positive detections of molecular markers was lower than that of phages infecting strain GA17. The ratio SOMCPH/GA17PH was shown also to be a good marker. These results provide information that will allow focusing measures for sanitation of the Bogotá River.

**Key words** | abattoir, *Bacteroides*, microbial source tracking, molecular markers, river, wastewater

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## INTRODUCTION

Pollution of water bodies with human and animal fecal wastes poses a clear risk to human health via waterborne pathogens (Ritter *et al*. 2002; WHO 2003; US EPA 2004). Among them, the most important ones are those following the fecal–oral route of infection, and within them, we found those causing acute gastroenteritis and diarrhea. According to the WHO (2008), infectious diarrhea is one of the main contributors to the global burden of disease with $4 \times 10^9$ estimated cases, and also being one of the leading causes of death, with a little more than two million deaths yearly. Human fecal contamination is considered more significant than animal fecal pollution in terms of human health risk. However, in some instances, fecal contaminants of animal origin can also have a significant impact on microbial water quality and the risk for human

health (WHO 2003). Moreover, recent studies indicate that the risk for human health associated with the fecal residues of different animals varies according to the species. Thus, risk associated with exposure to water impacted by cattle feces are greater than risks associated with exposure to water contaminated with gull, pig, or chicken feces (Soller *et al*. 2010).

Monitoring pathogens in water samples is unrealistic not only because it is costly and technically complex but also because pathogens are irregularly present in water bodies (Savichtcheva & Okabe 2006). For more than 100 years, the approach to evade this restraint has been to monitor for surrogate indicators, especially bacteria such as total coliforms, fecal coliforms, *Escherichia coli*, and enterococci (Leclerc *et al*. 2001; Tallon *et al*. 2005). These

microorganisms are frequently present in the lower intestine of homoeothermic animals including humans. Hence, the use of these indicators does not provide information about the host species that are the source of the fecal contamination in a given site. Fecal pollutants can reach water bodies through the direct discharge of fecal waste or untreated wastewater as well as secondary effluents from wastewater treatment works, combined sewer and sanitary overflows, animal farming activities, the wastewaters from abattoirs and the meat industry, and wildlife (US EPA 2004). The impact of each of these fecal sources to the pathogen load varies greatly among watersheds. Therefore, determining the source of fecal contamination in aquatic environments is essential not only to better estimate the health risks associated with fecal pollution, but also to facilitate measures to remediate contaminated waterways, and to determine legal responsibility for remediation.

Different approaches are being used to track fecal sources. The suitability of chemical markers has focused on the distribution pattern of chemicals such as caffeine, fragrance substances, fluorescent whitening agents, and fecal sterol isomers (Leeming *et al.* 1996; Standley *et al.* 2000; Hagedorn & Weisberg 2011). The use of eukaryotic mitochondrial DNA has also been explored to discern sources in fecal contaminated surface water (Caldwell *et al.* 2011). In addition, a variety of microorganisms has been considered for fecal source tracking purposes. The use of microorganisms for fecal source tracking is known as microbial source tracking (MST). MST is at present the most widespread approach for tracking the sources of fecal pollution.

Pathogenic and commensal microorganisms determined by culture-dependent and molecular methods, associated either with library-dependent or library-independent schemes, have been studied and reviewed as potential tools to identify human fecal sources. Library-dependent schemes are those associated with a database obtained in a given area (Simpson *et al.* 2002; Field & Samadpour 2007; Blanch *et al.* 2008; Hagedorn *et al.* 2011; Harwood *et al.* 2013). However, field studies using the available methods to track sources of fecal contamination have shown that a single marker or method usually is not enough to identify the source and that 'toolboxes' of a variable number of markers or methods are needed (Simpson

*et al.* 2002; Stewart *et al.* 2003; Santo Domingo *et al.* 2007; Blanch *et al.* 2008). Among others, one of the reasons for the imperfection of many of the methods available is that their sensitivity and specificity vary among different geographical areas (Hartel *et al.* 2002; Moore *et al.* 2005; Ebdon & Taylor 2006; Hansen *et al.* 2009; Reischer *et al.* 2013; Jofre *et al.* 2014).

Although data on the non-discriminating indicators tested here are available from Colombia and South America (Lucena *et al.* 2003), data on the adequacy of common microbial source tracers from these areas are non-existent or scarce. Here, we selected a number of the markers used elsewhere and evaluated their adequacy in the area of Bogotá. Bacteriophages infecting some strains of *Bacteroides* (Jofre *et al.* 2014), one of them isolated and selected in this work, and the sorbitol-fermenting bifidobacteria (SFBIF) (Blanch *et al.* 2006) were examined as culture methods. Targeted polymerase chain reaction (PCR) with *Bifidobacterium adolescentis* (ADO) and *Bifidobacterium dentium* (DEN) markers (Bonjoch *et al.* 2004; Blanch *et al.* 2006), *Bacteroidetes* marker HF183F (Bernhard & Field 2000), which are specific to human sources, and *Bacteroidetes* marker CF128F, which is specific to ruminant sources (Bernhard & Field 2000) were assayed as molecular methods. All these host-specific markers and *E. coli*, somatic coliphages (IAWPRC 1991) and bacteriophages infecting strain RYC2056 of *Bacteroides fragilis* (Jofre *et al.* 2014), which are markers of general fecal pollution, were determined in different human and animal wastewater sources and in the Bogotá River. Then, the numerical ratios of the concentrations of somatic coliphages/phages infecting selected strains of *Bacteroides* (Muniesa *et al.* 2012) and the ratio SFBIF/total bifidobacteria (TBIF) (Blanch *et al.* 2006) were also calculated as fecal source markers.

## MATERIALS AND METHODS

### Samples and sampling

Municipal raw sewage samples, assumed to contain mainly human fecal contamination, were collected at the influents of different municipal wastewater treatment plants (WWTPs) in Bogotá. Untreated wastewater was also

collected from various abattoirs, slaughtering mostly cows, in the Bogotá area. River water samples were gathered from either the Bogotá River or its tributaries. Eleven of the sampling sites were located in a section of the river unaffected by contaminating inputs from the Bogotá metropolitan area receiving the fecal contaminants from small towns, small slaughterhouses, and scaled-down cattle-raising activity. The other four sampling sites were downstream from the city of Bogotá where the waterway has received high inputs of municipal wastewaters and wastewater from three very large slaughterhouses. Water samples were kept at 4 °C and tested within 12 h post-collection.

### Escherichia coli enumeration

*Escherichia coli* was enumerated by membrane filtration using Chromocult coliform agar (Merck, Darmstadt, Germany) supplemented with antibiotics (*E. coli*/coliform selective supplement; Merck, Germany), and incubated for 24 h at 37 °C. The dark blue/purple colonies on the Chromocult agar were confirmed as *E. coli* by the addition of Kovac's reagent. Prior to filtration, the wastewater samples were diluted 1:10 in saline peptone, while the cattle manure samples were submitted to an elution step made according to Ebdon *et al.* (2007).

### Bifidobacterium enumeration

Human bifidobacteria sorbitol-fermenting agar (HBSA) (Mara & Oragui 1983) was used for the enumeration of the TBIF and SFBIF. Water samples were filtered through 0.45-μm pore size membranes (Millipore, Bedford, MA, USA) that were transferred onto HBSA according to the described procedures and incubated for 48 h at 37 °C under anaerobic conditions (GasPak; BBL, Hampshire, UK) with atmosphere generators of $CO_2$ (AnaeroGen; Oxoid, Basingstoke, UK). Yellow colonies were counted as SFBIF as described previously (Bonjoch *et al.* 2005).

### Bacteriophage enumeration

Bacteriophages were enumerated after filtration of the sample through low protein-binding hydrophilic

Polyethersulfone 0.22-μm pore size membrane filters (Millipore, Bedford, MA, USA) and the phages were enumerated as plaque forming units (PFUs) by the double agar layer technique. Somatic coliphages (SOMCPH) were enumerated on host strain *E. coli* WG5 in accordance with the ISO standardized procedure (Anonymous 2000). *Bacteroides* infecting bacteriophages were enumerated using the double agar layer plaque assay according to the ISO standardized method (Anonymous 2001). The host strains of *Bacteroides* tested were *Bacteroides fragilis* RYC2056 (Puig *et al.* 1999), *Bacteroides tethaiotaomicron* GA17 (Payan *et al.* 2005), both isolated in Spain; *Bacteroides tethaiotaomicron* HB13, isolated from fecal samples from Colombia (Payan *et al.* 2005), and *Bacteroides fragilis* CA8 (this work).

### Bacteriophage concentration

Bacteriophages were concentrated from 1 l of river water samples according to the method described by Méndez *et al.* (2004). $MgCl_2$ was added to the water samples to a final concentration of 0.05 mol l$^{-1}$. Briefly, the sample was then passed through an acetate-nitrate cellulose ester membrane filter (Millipore, Bedford, MA, USA), 0.22-μm pore size, and 47-mm diameter, at a filtration rate of about 2 l h$^{-1}$. Then, the membrane was cut into eight fragments and placed in a glass flask containing 5 ml eluting solution (1% beef extract, 0.05 mol l$^{-1}$ NaCl and 3% Tween 80). The flask was placed in an ultrasound-cleaning bath for 4 min and the eluted bacteriophages were counted as described previously.

### Isolation of new host strains for phages infecting *Bacteroides*

Samples of municipal wastewater were examined for new *Bacteroides* host strains specific to bacteriophages of human origin. The isolation of presumptive *Bacteroides* strains, the preparation of phage suspensions, and the screening of specific strains was performed as in Payan *et al.* (2005). First, black colonies with a black or dark halo grown on *Bacteroides* bile esculine agar were isolated as presumptive *Bacteroides*. Then, the isolates that were Gram-negative rods growing only under strict anaerobic

conditions, propagating satisfactorily in BPRM (*Bacteroides* phage recovery medium) broth (Anonymous 2001), detecting bacteriophages in phage suspensions from municipal wastewater and failing to detect phages from abattoir wastewaters were selected as *Bacteroides* host strains potentially able to detect preferably bacteriophages present in waters contaminated with human fecal residues.

The CA8 strain, which was a Gram-negative rod, showing strict anaerobic growth, adequate growth in BPRM, and positive detection of phages in municipal wastewater and not in slaughterhouse wastewater was characterized by sequencing the 16S RNA gene. The DNA of CA8 strain was extracted and purified with Wizard SV Gel Kit and PCR Clean-up System (Promega, Madison, WI, USA) was used as template. PCR amplification of the 16S rRNA gene was performed according to Weisburg *et al.* (1991). The primers used were Upper Bact 16S (GCTACCTTCTTACGACTT) and Lower Bact 16S (GAGTTTGATCCTGGCTC) (Weisburg *et al.* 1991) with an annealing temperature of 50 °C. A 5 µl volume of each PCR product was analyzed by agarose (1.5%) gel electrophoresis, and bands were visualized by staining with ethidium bromide. The 1,488-kDa PCR products obtained were used directly for sequencing. Sequencing was performed with the Sanger method (Sanger & Coulson 1975). Searches for homologous DNA sequences in the EMBL and GenBank database libraries were performed with the BLAST tool (http://www.ncbi.nlm.nih.gov).

## Bacterial DNA extraction from water samples

DNA was directly extracted from 250 µl of slaughterhouse and wastewater samples using the QIAamp®DNA Blood Mini Kit (Qiagen GmbH, Hilden, Germany) in accordance with the manufacturer's instructions. The DNA was eluted in 100 µl of the elution buffer provided by the manufacturer.

## *Bacteroidetes* group detection

Specific PCR primers, HF183F and CF128F, designed by Bernhard & Field (2000) were used to discriminate *Bacteroidetes* from human and ruminant sources of fecal contamination, respectively. These primers were designed as a function of the uncultured markers obtained from an analysis of the *Bacteroidetes* communities in feces using the terminal restriction fragment length polymorphism (T-RFLP) technique. The PCR amplification was carried out according to Ballesté *et al.* (2010). Each 25 µl PCR mixture contained the following: 1X buffer (Kit TucanTaq, CorpoGen, Colombia), each primer at a concentration of 0.2 µM, 0.625 U of *Taq* polymerase (Kit TucanTaq, CorpoGen, Colombia), 1.5 mM MgCl₂, 0.2 mM each nucleotide, and 1 µl of the extracted DNA. The reaction was performed in an MJ Research PTC-100 thermal cycler. The conditions were as follows: an initial denaturation at 94 °C for 2 min, 35 cycles consisting of 94 °C for 1 min, a suitable annealing temperature for every marker for 1 min (62 °C for the ruminant marker and 63 °C for the human marker) and 72 °C for 1.5 min, followed by a final 7 min extension at 72 °C. The reaction mixture was cooled down to 4 °C. The amplicons were separated after electrophoresis on 3% agarose gels and visualized with ethidium bromide staining.

## *Bifidobacterium adolescentis* and *B. dentium* detection

The procedure consisted of two amplification steps. First, a PCR was performed using *Bifidobacterium* genus-specific primers, lm26 and lm3 (Kaufmann *et al.* 1997). Then, PCR products were used for PCR with sets of primers specific for *Bifidobacterium* spp. ADO-DEN multiplex PCR was used to detect *B. adolescentis* and *B. dentium* in all samples as described previously (Bonjoch *et al.* 2004); briefly, 1X buffer (Kit TucanTaq, CorpoGen, Colombia), the species-specific primers for *B. adolescentis* at 0.25 µM concentration (Matsuki *et al.* 1999), the species-specific primer for *B. dentium* at 0.50 µM concentration, 0.04 U of *Taq* polymerase (TucanTaq, CorpoGen), 0.5 mM MgCl₂, 0.2 mM each nucleotide, and 1 µl of the extracted DNA. PCR parameters in the MJ Research PTC-100 thermal cycler were the following: an initial denaturation at 94 °C for 5 min, 35 cycles consisting of 94 °C for 20 sec, a suitable annealing temperature for 20 sec at 55 °C and 72 °C for 30 sec, followed by a final 5 min extension at 72 °C. The reaction mixture was cooled down to 4 °C. The amplicons were separated after electrophoresis on 3% agarose gels and visualized with ethidium bromide staining. Positive controls were performed using *B. adolescentis* DSM 20083 and *B. dentium* DSM 20084.

## Statistics

Statistical computations and tests were performed using Statgraphics Plus software (version 5.1; Rockville, MD, USA). Differences were considered significant at $P < 0.05$ as determined by the appropriate comparative test.

## RESULTS AND DISCUSSION

### Isolation of a *Bacteroides* host strain

Thirty-six black colonies on *Bacteroides* bile esculine agar were selected from two samples of municipal sewage. All the corresponding isolates were Gram-negative rods, but only 15 were strict anaerobes. Six of them grew in BPRM and four detected bacteriophages in municipal wastewater. One of them, termed CA8, detected phages in five of five municipal sewage samples with values expressed in $\log_{10}$ units ranging from 2.0 to 4.8 PFUs per 100 ml, averaging 3.6 $\log_{10}$ units. In contrast, phages were not detected by strain CA8 in samples of slaughterhouse wastewaters tested.

The BLASTn analysis of the sequence (deposited in GenBank with accession numbers BankIt1777297 Seq1 KP188833) of the 1,488-kDa fragment amplified from DNA of strain CA8 showed a 97% similarity with *Bacteroides fragilis* 16S rRNA X83940.1 and *B. fragilis* strain NCTC 9343.

This strain detects lower numbers of phages than other *Bacteroides* host strains such as *Bacteroides tethaiotaomicron* GA17 and HB13 do in the area of Bogotá and consequently it is of little practical interest. However, its isolation confirms that with little effort it is possible to isolate host strains for phages of *Bacteroides* discriminating between human and animal fecal pollution in a given geographical area, as shown before (Payán *et al.* 2005; Jofre *et al.* 2014).

### Markers at sources

*Escherichia coli*, SOMCPH, phages infecting strain RYC2056 of *Bacteroides fragilis* and TBIF, which are markers of general fecal pollution, were present in all the samples tested, independently of their origin (Table 1). As well, SFBIF were detected in all the samples tested herein (Table 1). This results regarding SFBIF are in agreement with results reported in Blanch *et al.* (2008), but not with those reported in Mara & Oragui (1983), where they were reported to be mostly present in samples of human origin. In contrast, the culturable markers able to discriminate fecal sources, this is bacteriophages infecting strains GA17, HB13, and CA8, were found in all samples of human origin but in none of the samples of animal origin

**Table 1** | Concentrations (in $\log_{10}$ units per 100 ml) of cultivable markers in heavily contaminated water samples (municipal WWTP and wastewater from abattoirs)

|  | Municipal WWTP ($n = 5$) | | | Abattoir wastewater ($n = 4$) | | |
|---|---|---|---|---|---|---|
|  | Average | Range | % Pos | Average | Range | % Pos |
| *E. coli* | 6.6 | 5.7–7.0 | 100 | 6.9 | 6.4–7.7 | 100 |
| SOMCPH | 5.7 | 5.1–5.9 | 100 | 7.0 | 6.6–7.7 | 100 |
| RYC2056PH | 4.1 | 3.7–4.4 | 100 | 2.0 | 2.0–2.1 | 100 |
| GA17PH | 4.5 | 4.0–5.6 | 100 | <2.0 | <2.0 | 0 |
| HB13PH | 3.6 | 2.5–4.0 | 100 | <2.0 | <2.0 | 0 |
| CA8PH | 3.6 | 2.0–4.8 | 100 | <2.0 | <2.0 | 0 |
| SFBIF | 5.9 | 4.8–7.0 | 100 | 4.1 | 2.7–5.8 | 100 |
| TBIF | 6.5 | 5.4–7.5 | 100 | 5.0 | 3.8–6.5 | 100 |
| Ratio SFBIF/TBIF | 0.26 | 0.15–0.30 | – | 0.11 | 0.05–0.17 | – |
| Ratio (in $\log_{10}$ units) SOMCPH/GA17HP | 1.24 | 0.1–1.9 | – |  | >4.6– > 5.2 | – |

RYC2056PH, GA17PH, HB13PH, CA8PH are bacteriophages infecting strains RYC2056, GA17, HB13, and CA8 of *Bacteroides*, respectively; *n* is the number of samples; SFBIF/TBIF: ratio of sorbitol-fermenting/TBIF; other abbreviations are as in the 'Materials and methods' section.

tested. The highest values were detected by strain GA17. PFU values counted on strain GA17 ranged from 4.0 to 5.6 $\log_{10}$ units per 100 ml of municipal wastewater. Despite being isolated in Spain, strain GA17 performed better than strains HB13 and CA8 isolated from Colombian raw sewage samples. The ratios between the concentration of somatic coliphages and bacteriophages detected by strain GA17 of *Bacteroides* expressed in $\log_{10}$ units were lower than 2 in the human sources and greater than 4.6 in animal sources. These differences are statistically significant (ANOVA (analysis of variance) $P < 0.05$) and similar to those described elsewhere overseas (Muniesa *et al.* 2012). The SFBIF/TBIF ratios ranged from 0.15 to 0.30 in human sources and from 0.05 to 0.17 in animal sources. These values in human sources are lower than values reported elsewhere, whereas values in animal sources are higher (Blanch *et al.* 2006).

Regarding the molecular markers (Table 2), they all provided absolute specificity. Indeed, none of them was found in the non-corresponding source samples. Regarding sensitivity, ADO and HF183F were identified in all the corresponding source samples, whereas CF128F failed to be detected in 25% samples of slaughterhouse and DEN in 60% of municipal sewage samples. These results indicate that regarding specificity, all these molecular markers comply with the expected results in highly contaminated water samples in Colombia.

## Markers in Bogotá River samples

The values of markers with quantitative results are displayed in Table 3. They clearly show the high impact of the fecal inputs contributed by the conurbation of Bogotá. Upstream from Bogotá, only *E. coli*, SOMCPH, and TBIF were

detected in all the samples, with maximum values expressed in $\log_{10}$ units per 100 ml of 3.5, 3.4, and 4.4, respectively. All the values were between three and four orders of magnitude lower than those detected in raw municipal sewage and abattoir wastewater samples. SFBIF were detected in 45% of samples, and the ratio SFBIF/TBIF ranged from <0.25 to 0.6. In contrast, bacteriophages infecting *Bacteroides* strains RYC2056, which is non-discriminating, GA17 and HB13 were only found in one out of eleven samples when only 10 ml was analyzed. In the positive sample, the ratio SOMCPH/GA17PH was 1.2 $\log_{10}$ units, indicating that human fecal contamination is predominant. In the rest of the samples of this group, the values of the ratio ranged from >1.0 to >2.4. These values do not provide sufficient information about the source according to available information (Jofre *et al.* 2014). No phages infecting CA8 were detected at concentrations >10 PFUs per 100 ml in this set of samples.

To gain insight on the limitations of the method in samples with intermediate to low concentrations of fecal indicators, bacteriophages were concentrated from 1 l volumes taken from sampling points where phages infecting *Bacteroides* were not detected in 10 ml volumes. Values of the parameters tested in these samples are shown in Table 4. The number of positive detections increased, thus showing that testing phages in 1 l of river water with a very simple method is significantly improving the sensitivity of the procedure. With these data, the ratio SOMCPH/GA17PH was calculated in three samples, showing two values of 2.7, which indicates the presence of human contamination as being predominant (Muniesa *et al.* 2012). The third sample gave a value greater than 3.5, which is unlikely to indicate the predominance of human fecal contamination (Jofre *et al.* 2014).

Regarding molecular markers, the only positive detections (Table 2) were ADO and DEN. They were found in a single sample, the same one in which phages infecting *Bacteroides* strains GA17 and HB13 were detected. In addition, this sample was one of the few samples with *E. coli* above $10^3$ colony forming units (CFUs) per 100 ml. In this particular sample, all the markers, except HF183F and phages infecting CA8, indicate a human origin of fecal contamination. None of the molecular markers was detected in the rest of the samples in this set of samples.

**Table 2** | Detection of molecular markers in different kinds of water

|  | Municipal WWTP | Abattoir wastewater | Bogotá River upstream Bogotá | Bogotá River downstream Bogotá |
|---|---|---|---|---|
| ADO | 100 (5/5) | 0 (0/4) | 9.0 (1/11) | 25 (1/4) |
| DEN | 40 (2/5) | 0 (0/4) | 9.0 (1/11) | 25 (1/4) |
| HF183F | 100 (5/5) | 0 (0/4) | 0 (0/11) | 50 (2/4) |
| CF128F | 0 (0/5) | 75 (3/4) | 0 (0/11) | 50 (2/4) |

In brackets, the positive samples over the number tested.

**Table 3** | Concentrations (in $\log_{10}$ units per 100 ml) of cultivable markers in the Bogotá River

| | Upstream Bogotá ($n = 11$) | | | Downstream Bogotá ($n = 4$) | | |
|---|---|---|---|---|---|---|
| | Average | Range | % Pos | Average | Range | % Pos |
| *E. coli* | 3.0 | 2.6–3.5 | 100 | 5.8 | 4.6–6.7 | 100 |
| SOMCPH | 2.6 | 2.0–3.4 | 100 | 4.4 | 3.9–4.9 | 100 |
| RYC2056PH | – | <1.0–1.7 | 9.0 | 2.5 | 1.8–3 .4 | 100 |
| GA17PH | – | <1.0–1.6 | 9.0 | 2.5 | 1.6–3.4 | 100 |
| HB13PH | – | <1.0–1.5 | 9.0 | – | <1.0–3.6 | 75 |
| CA8PH | – | <1.0–1.3 | 9.0 | – | <1.0–3.0 | 25 |
| SFBBIF | – | <3.0–3.4 | 45.0 | 5.7 | 4.5–6.4 | 100 |
| TBIF | 3.5 | 3.0–4.4 | 100 | 6.5 | 5.4–7.2 | 100 |
| Ratio SFBIF/TBIF | | >0.3–0.5 | 45.0 | 0.16 | 0.11–0.22 | 100 |
| Ratio (in $\log_{10}$ units) SOMCPH/GA17PH | | >1.0– >2.4 | 9.0 | 1.8 | 1.4–2.2 | 100 |

RYC2056PH, GA17PH, HB13PH, CA8PH are bacteriophages infecting strains RYC2056, GA17, HB13, and CA8 of *Bacteroides*, respectively; *n* is the number of samples; SFBIF/TBIF: ratio of sorbitol-fermenting/TBIF; other abbreviations are as in the 'Materials and methods' section.

**Table 4** | Concentrations (in $\log_{10}$ units per liter) of culturable markers in samples upstream of the Bogotá conurbation after bacteriophage concentration from 1 liter samples

| | Sample 1 | Sample 2 | Sample 3 |
|---|---|---|---|
| *E. coli* | 3.9 | 3.8 | 4.0 |
| SOMCPH | 3 | 3.5 | 3.9 |
| RYC2056PH | 2 | 1.9 | 2.1 |
| GA17PH | 1.3 | <0 | 2.2 |
| HB13PH | 2.2 | 1 | <0 |
| Ratio (in $\log_{10}$ units) SOMCPH/ GA17PH | 2.7 | >3.7 | 2.7 |

RYC2056PH, GA17PH, HB13PH, CA8PH are bacteriophages infecting strains RYC2056, GA17, HB13, and CA8 of *Bacteroides*, respectively; other abbreviations are as in the 'Materials and methods' section.

In contrast, and due to the great impact caused by the input of the city of Bogotá with depurated or non-depurated municipal sewage and the wastewater of several abattoirs, the number of parameters with positive detection was significantly greater ($P < 0.05$, ANOVA) than those observed in samples collected upstream of the Bogotá conurbation (Table 3). Values of *E. coli* rose to over $10^6$ CFUs per 100 ml in the city area and decreased to $4 \times 10^4$ at some distance downstream. Bacteriophages infecting strain GA17 were detected in all samples, with values ranging from 1.6 to 3.4 $\log_{10}$ PFUs per 100 ml and the ratios SOMCPH/GA17PH ranged from 1.4 to 2.2, which are characteristic values of human source contamination. Phages infecting HB13 and CA8 were not detected in one of the samples, confirming a better performance of strain GA17. In contrast, values of the ratio SFBIF/TBIF ranged from 0.11 to 0.22, which are more typical of animal fecal contamination, thus confirming the poor performance of this ratio observed in wastewaters. Regarding the molecular markers, ADO and DEN were detected in only one sample that had more than $10^6$ *E. coli* CFUs per 100 ml. HF183F was detected in two of the samples that were also those with *E. coli* exceeding $10^6$ CFUs per 100 ml. CF128F was also detected in the sample where all the other molecular markers were detected.

## CONCLUSIONS

With the exception of the ratio SFBIF/TBIF, the markers chosen are applicable for MST in the area studied. To our knowledge, this is the first study in South America providing information on such a number of MST markers. The bacteriophages infecting discriminating hosts of *Bacteroides*, particularly those infecting strain GA17 of *Bacteroides tethaiotaomicron*, as well as the molecular markers, show a good specificity and sensitivity in the municipal sewage and slaughterhouse wastewater. Only the ratio SFBIF/ TBIF failed regarding specificity.

When these MST markers were evaluated in samples from a contaminated river with concentrations of *E. coli* below $10^3$ all methods showed low sensitivity and their detection will require concentration. Concentrating phages in a 1 l sample for host-specific phages infecting *Bacteroides* is sufficient to enable the calculation of the ratio SOMCPH/GA17PH that seem to be one of the best predictive markers, according to the data reported abroad (Muniesa *et al.* 2012).

Findings reported herein contribute to upgrade the current MST methodology in the region, and very likely by extension in South America, and hence to the implementation of management practices of water resources in a way aimed to decrease the health risks associated with fecal contamination. In addition, data provided herein will help to reduce health risks associated with water by allowing policy-makers to better focus correcting measures aimed at improving the sanitation of the Bogotá River, which is the main source of water for human consumption and irrigation for the most populated region of the country.

## ACKNOWLEDGEMENTS

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